Structure
Previews

Variations on Glutamate Binding in Channel-Gated Receptors

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In this issue of *Structure*, Lomash and colleagues report on the crystal structure and ligand binding properties of a primitive eukaryote glutamate-gated ion channel: AvGluR1.

In the mammalian central nervous system (CNS), nerve impulse is rapidly transmitted by a chemical neurotransmitter that is released from one cell and activates receptors on the next cell, allowing the message propagation. Many of the CNS pathologies are due to an impairment of transmission at this synaptic junction. Accordingly, large efforts are devoted to the investigation of receptors involved in this event, as they represent valuable therapeutic targets. Glutamate is the major excitatory neurotransmitter and activates post-synaptic membrane receptors. When binding to these receptors, glutamate triggers the opening of an associated ion channel. Such ionotropic glutamate receptors (iGluRs) have been identified in prokaryotes, eukaryotes, and even plants, yet with structural and functional differences (Figure 1).

They have been intensively studied, and some major structural data have been published. It is proposed that eukaryotic iGluRs evolved from prokaryotic receptors, but the evolutionary link still needs to be characterized. In this issue of *Structure*, Lomash et al. (2013) shed light on this matter and report on the structure and agonist, particularity of a primitive eukaryotic iGluR named AvGluR, that has been recently discovered (Janovjak et al., 2011).

Glutamate receptor ion channels are tetramers of modular protein subunits composed of a ligand binding domain (LBD) and a transmembrane domain (TMD) that forms part of the ion channel pore. The LBD is formed of two globular domains linked by a flexible hinge and belongs to the same structural family as some bacterial periplasmic proteins (periplasmic binding protein-like II in the SCOP classification). The glutamate binding site is located in the cleft of this clamshell-shaped LBD. Whereas all iGluRs share these common attributes, notable differences are also found between prokaryotes and eukaryotes. It is probable that the second type of receptors, which are more complex, evolved from the first bacterial ones. They hold three transmembrane segments with a cytoplasmic C-terminal domain (CTD), while the bacterial prokariotes have two (Figure 1). Another major structural difference is the presence of an additional extracellular amino terminal domain (ATD) that modulates the activity of the receptor. This ATD domain adopts a fold that belongs to the bacterial periplasmic proteins like the LBD but from a different family (periplasmic binding protein-like I in the SCOP classification) (Figure 1). According to crystal structures of the LBD that are available for bacterial GluR0 (Protein Data Bank [PDB] 1II5) and vertebrate AMPA (PDB 3TDJ and 3KGC), kainate (PDB 2XXR), and NMDA (PDB 2A5S) receptors, glutamate binds in an extended or folded conformation in prokaryotic and eukaryotic iGluRs respectively (Figure 2). Interestingly, residues binding the proximal amino acid functions of glutamate form a binding motif that is conserved across species (Figure 2). In contrast, the asparagine residue that secures an extended glutamate conformation through the binding of the distal acidic function in bacteria (e.g., GluR0, Figure 2A) has evolved to a glutamate residue in vertebrates. In this case, a probable repulsion between the negative charges carried by this residue and the ligand

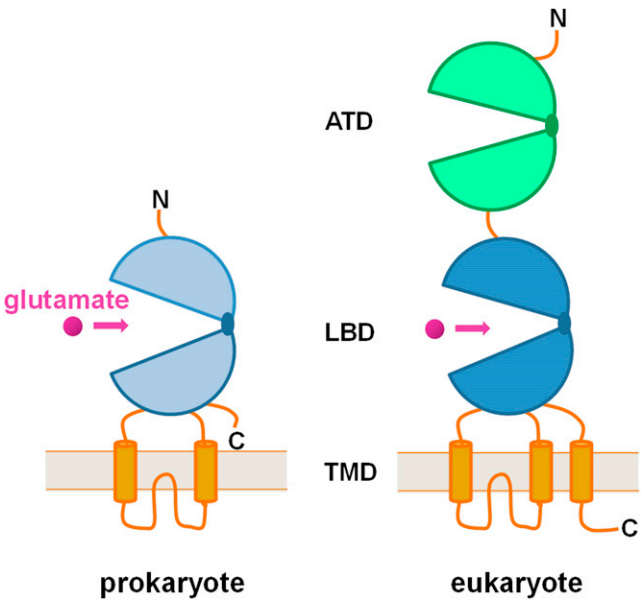

Figure 1. Schematic Illustration of the Subunit Structure of Bacterial and Eukaryote iGluRs
Glutamate receptor subunits have a modular structure composed of one (prokaryotes) or two (eukaryotes) large extracellular domains, the LBD (blue) and the amino terminal domain (ATD, green), a TMD (orange) that forms part of the ion channel pore, and an intracellular CTD.

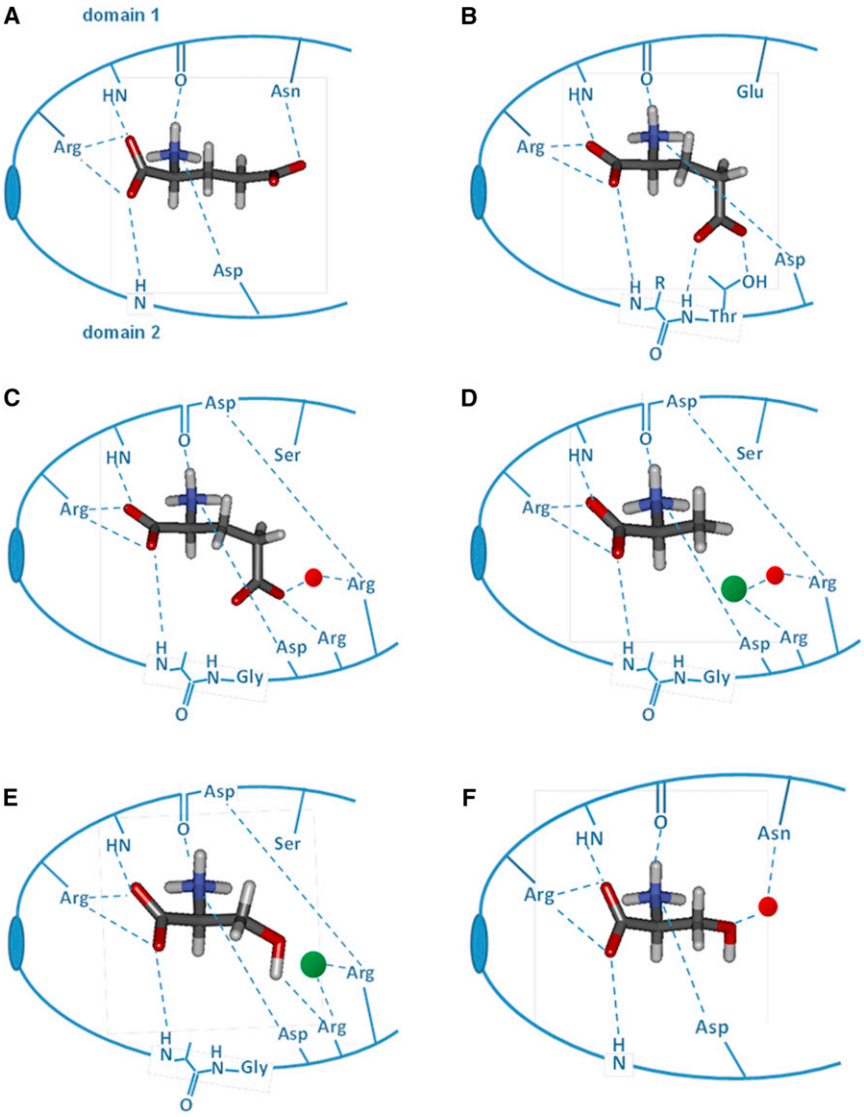

Figure 2. Schematic Representation of the LBD and Bound Agonist from Ionotropic Glutamate Receptor Crystal Structures

(A–C) Comparison of ligand interactions of bacteria (GluR0; A), vertebrate (AMPA, kainate, and NMDA; B), and AvGluR1 (C) iGlu receptors in their active conformation where glutamate is trapped in a closed form of the clamshell LBD.

(D) Shows AvGluR1 LBD bound with alanine; one of the six water molecules surrounding the agonist is displayed (red sphere). A chloride ion (green sphere) acts as a surrogate of the γ-carboxylate of glutamate.

(E and F) Comparison of the binding of serine to AvGluR1 (Lomash et al., 2013; E) and GluR0 (Mayer et al., 2001; F) highlights that the serine OH group makes a water-mediated hydrogen bond in GluR0 (F), whereas in AvGluR1 (E) it is via a chloride ion. The agonists are displayed in sticks with gray carbons, red oxygens, blue nitrogens, and white hydrogens and bind to both domains of the LBD. Hydrogen bonds are shown as dashed lines.

causes glutamate to fold (Figure 2B). For these vertebrate receptors, a threonine in domain 2 of the LBD is found to bind the distal carboxylic group of glutamate (Figure 2B). Differences are also found in the functional and pharmacological characteristics of the two types of iGluRs. The bacterial iGluRs are K^+-selective channels and are activated by several amino acids besides glutamate, as vertebrates are nonselective cation channels and are only activated by glutamate and specific synthetic agonists. It is now of high interest to discover and characterize primitive eukaryotic iGluRs that will provide insights into the evolution of these essential receptors. Janovjak et al. (2011) have recently discovered the primitive AvGluR1, which may be viewed as an "intermediate" receptor. In this issue of *Structure*, Lomash et al. (2013) report on its structure, which allows interpretation of its pharmacological profile.

AvGluR1 is found to hold attributes from both bacterial and vetebrate iGluRs. It shares an ATD and three membrane-spanning segments with eukaryotic receptors (Figure 1). The AvGluR1 LBD packing is the same as that found in prokaryotic iGluRs, but with a low affinity for dimer assembly like eukaryote iGluRs. A structure-based phylogenetic analysis revealed that the AvGluR1 LBD most closely resembled that of prokaryotic iGluRs, although bound glutamate adopts the same folded conformation as in NMDA, AMPA, and kainate receptors and not the extended conformation found in the prokaryote LBD of iGluR structures (Figure 2). Analysis of residues interacting with glutamate/aspartate shows that the proximal binding motif is conserved in AvGluR1 but not the distal set of residues binding the γ-(or β-) carboxylate, which is bound to two basic residues: R676 and R702 from domain 2 (Figure 2C). With agonists that lack an acidic group on their side chain, it was observed that a chloride ion acts as a surrogate of the carboxylic group (Figures 2D and 2E). This unusual pattern provides an explanation for the surprising promiscuous selectivity of AvGluR1 agonists compared to the stringent selectivity for dicarboxylic amino acids of vertebrate iGluRs. Indeed, AvGluR1 is activated by alanine, serine, cysteine, methionine, and phenylalanine in addition to glutamate and aspartate. The LBD crystal structures of complexes with these various amino acids show how a chloride ion can fit in a dense hydrogen bond network instead of the distal acidic group (Figure 2D). Interestingly, serine has been crystallized bound to AvGluR1 (Figure 2E) (Lomash et al., 2013) but was also previously bound to bacterial GluR0 (PDB 1IIT; Figure 2F) (Mayer et al., 2001). It appears that when bridging a neutral residue (e.g., Asn, Figure 2F), a water molecule

may act as a surrogate; when bridging a positively charged residue (e.g., Arg, Figure 2E), a negatively charged ion is required. Checking all vertebrate glutamate receptors that include iGluRs but also metabotropic glutamate receptors (mGluRs) that are G protein coupled receptors, basic residues binding the distal γ-carboxylate of glutamate are only found among mGluRs. Thus, one may wonder if AvGluR1 may be considered as an evolution stage of iGluRs or a divergent branch, leaving open the question of how bacterial iGluRs evolved to AMPA, kainite, and NMDA receptors.

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A "New Twist" on RGS Protein Selectivity

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G protein-coupled receptors mediate a wide array of physiologic stimuli and, together with their regulators such as RGS2, are essential components of cellular signaling and function. RGS2 is a selective inhibitor of the Gαq class of α subunits. In this issue of *Structure*, Nance and colleagues provide structural insight into the features of RGS2 that mediate its potent and selective regulation of Gαq function.

Signal transduction by G protein-coupled receptors (GPCRs) controls a wide array of physiological processes in eukaryotic organisms. Thus, a protein that can selectively regulate the magnitude, kinetics, and fidelity of signaling via GPCRs is critically important for the integration and control of diverse cellular processes such as nociception, cardiac pacemaking, cell migration, and apoptosis. Regulator of G protein signaling (RGS) proteins are important regulators of GPCR signaling and, therefore, cell physiologic processes. There are >35 members of the RGS protein superfamily that have been identified in the human genome. These proteins may be classified into one of several subfamilies (RZ, R4, R7, R12, RA, GEF, GRK, SNX, and D-AKAP) depending on their architectural organization. Each RGS protein is typified by an ~120 amino acid RGS homology (RH) domain. In the case of roughly 50% of these proteins, the RH domain exhibits guanosine triphosphatase (GTPase)-activating protein (GAP) activity toward one or more G protein α subunits and thus serves to regulate GPCR signaling (Berman et al., 1996). From a biochemical perspective, these RH domains exert their catalytic function by binding to the three flexible switch domains (SwI–III) on the Gα subunit in a manner that stabilizes the transition state-like conformation for GTPase hydrolysis.

Since the discovery of RGS proteins in the mid-1990s, several investigators have explored the possibility that each RGS family member may selectively regulate a specific subset of biologic signaling pathways. Such pathway specificity for an RGS protein might be based on its cell-type restricted gene expression or even its geographical compartmentalization within specific effector cells. Both of these elements are in place for RGS9-1, a terminator of phototransduction that is mediated via rhodopsin and Gαt in the visual system. RGS9-1 expression is essentially restricted to rod and cone cells on the retina, and the RGS9-1 protein can be selectively targeted to the rod-outer segments via its interaction with the RGS9-specific anchoring protein (Cowan et al., 1998; Martemyanov et al., 2003). Together, these characteristics make it a potent and selective regulator of phototransduction events. Many RGS proteins, however, do not show highly restricted gene expression patterns or obvious intracellular compartmentalization, suggesting the need for further refinement of signaling selectivity at the level of the individual RGS protein-Gα subunit pairs. Indeed, some RGS protein family members show remarkable binding selectivity and regulation of specific Gα binding partners. For example, members of the R7 (RGS6, RGS7, RGS9, and RGS11) and R12 (RGS10, RGS12, and RGS14) RGS protein subfamilies appear to be specific for Gαi/o subunits (Cho et al., 2000; Soundararajan et al., 2008). By contrast, one member of the R4 subfamily, RGS2, appears to shows a strong biochemical preference for interaction with Gαq (Heximer et al., 1997). A study by Nance et al. (2013) in this issue of *Structure* identifies additional sets of structural determinants that help to mediate the biochemical selectivity of RGS2 for Gαq.

Previous work by our group and others pointed to the importance of three unique residues in RGS2 (relative to other R4 group proteins) as key determinants of its preference for interaction with Gαq over Gαi (Heximer et al., 1999, Kimple et al., 2009). The replacement of all three of these residues with the corresponding residues from RGS4 was sufficient to